

12 **EUROPEAN PATENT APPLICATION**

21 Application number: 84303090.9

22 Date of filing: 08.05.84

51 Int. Cl.³: **G 01 N 33/54**
C 12 Q 1/68
//C07F17/02, C07D473/08

30 Priority: 05.05.83 GB 8312263
05.05.83 GB 8312259
05.05.83 GB 8312265
21.09.83 GB 8325316
16.12.83 GB 8333650
16.12.83 GB 8333651
19.01.84 GB 8401399
29.02.84 GB 8405262
29.02.84 GB 8405263

43 Date of publication of application:
14.11.84 Bulletin 84/46

84 Designated Contracting States:
BE CH DE FR GB IT LI NL SE

71 Applicant: **Genetics International, Inc.**
50 Milk Street Fifteenth Floor
Boston, MA 02109(US)

72 Inventor: **Hill, Hugh Allen Oliver**
9 Clover Close
Oxford OX2 9JH(GB)

74 Representative: **Clifford, Frederick Alan et al,**
MARKS & CLERK 57/60 Lincoln's Inn Fields
London WC2A 3LS(GB)

54 Assay techniques utilising specific binding agents.

57 This specification discloses assay techniques and especially those for the detection measuring and monitoring of complex, usually biologically-arising, molecules or part-molecular structure often in complicated admixture with other similar molecules. The techniques of the invention utilise specific binding agents, i.e.g complex molecular species which have a specific reaction, usually based on electrostatic or hydrophobic forces, with one or more active sites on a given type of molecule only.

One limb of this invention is concerned with immunoassay techniques for ligands and analytes to be carried out in vivo or in vitro. It should be noted that these ligands include species which are not only *per se* antigenic, but may be made so by crosslinking them to a suitable carrier.

Another limb of the present invention is concerned with; firstly techniques for investigating DNA or RNA sequencing, and secondly with other types of reactions in which specific, non-immunological binding occurs. The specification further discloses equipment or materials useful in the above techniques, either as such or possibly as usable coordinated kits of parts.

The specification particularly discloses, an assay system comprising

- (a) a ligand-antiligand pair or analyte-specific binding agent pair capable of specifically binding together, (T.A)
- (b) a mediator (F) enzyme (GOD) and corresponding substrate (GLUCOSE) triplet, whereby conversion of the sub-

trate into a product by the enzyme generates electrical charge which is transferred *via* the mediator to an electrode;

wherein at least some of one of the pair and at least some of one of the triplet are chemically cross-linked (GOD-T₄), so as to inhibit at least a part of the said charge transfer when the ligand and antiligand pair or analyte-specific binding agent pair are bound together and,

wherein the addition of either one of the said pair or one of the said triplet changes the rate of charge transfer to the electrode.

